Bioorganic & Medicinal Chemistry Letters 25 (2015) 597–601

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-biofilm activities of dihydro-pyrrol-2-one derivatives on *Pseudomonas aeruginosa*

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ARTICLE INFO

Article history:
Received 2 October 2014
Revised 17 November 2014
Accepted 5 December 2014
Available online 13 December 2014

Keywords:
DPO derivatives
Tandem reaction
Bacterial biofilm
Mannitol dehydrogenase
Extracellular DNA

ABSTRACT

Biofilm formation is an important reason for bacterial resistance to antimicrobials. Many compounds with dihydro-pyrrol-2-one (DPO) have antibacterial effects. It is prospective to base on DPO skeleton to design new compounds for biofilm inhibition. DPO was designed by a novel method of tandem cyclization between ethyl glyoxalate and amines, the series of DPO derivatives were synthesized by change of the amines. Their activities were evaluated by the inhibition of biofilm in *Pseudomonas aeruginosa*. The interaction of DPO derivatives with mannitol dehydrogenase (MDH) or extracellular DNA (eDNA) in the biofilm was simulated by molecular docking to reveal possible mechanism. 19 new DPO derivatives were synthesized and identified, 15 of them had antibacterial activities, but only 5 of them had more than 50% inhibition on biofilm of *P. aeruginosa* at 50 μg/mL. The MDH activity and eDNA content in biofilm decreased significantly after treatment of the DPO derivatives in concentration dependence. The simulation reveals that strong interaction exists between the five DPO derivatives and MDH or eDNA, which are involved in anti-biofilm mechanism. The synthetic method of DPO derivatives is practical to provide effective anti-biofilm agents for *P. aeruginosa*, and they take effect through inhibition on MDH and eDNA of biofilm.

Dihydro-pyrrol-2-one (DPO), a key building block for constructing a variety of bioactive ingredients or intermediates, widely exists in pheromone, alkaloids, steroids, heme, chlorophyll and other compounds, and it has very strong antibacterial and other pharmacological activities.[1] The inherent ring-conjugate system and chirality of DPO contribute to its high region-selectivity and stereo-selectivity in chemical reaction,[2] which are conducive to the functionalization of specific sites in the molecules.[3] Therefore, DPO synthesis plays an important role in rational drug design and drug screening.

Many methods exist today for the synthesis of DPO derivatives, including the multistep method,[4] the two component method,[5–7] the three component method[8,9] and the multi-component method.[10,11] The first method involves a cumbersome and inefficient process of separation and purification, the second and the third methods need the complex substrates, which are difficult to synthesize, and the last one requires many kinds of raw materials. We designed a new tandem reaction with two simple components (aldehydes and amines) to synthesize DPO derivatives.

Pseudomonas aeruginosa is the major pathogenic bacterium for pulmonary nosocomial infection, and it easily acquire drug resistance, leading to the deficiency of therapeutic effects.[12] Biofilm formation is an important reason for bacterial resistance and diseases recurrence.[13] It is meaningful to find out effective anti-biofilm agents for therapy of *P. aeruginosa* induced infections. DPO has small molecular structure, may play a role in anti-biofilm with strong permeability. It is prospective to design and screen out anti-biofilm agents with DPO skeleton based on new synthetic methods.

The synthetic route of DPO derivatives was designed as shown in Figure 1. They were synthesized by ethyl glyoxalate and amines with anhydrous sodium sulfate as water absorbent, Pd $(TFA)_2$ as catalyst and toluene as solvent. Each substrate was repeated five times to ensure the synthesis yield.

Conditions for the palladium-catalyzed cascade cyclization of ethyl glyoxalate with *p*-anisidine were optimized in our previous research,[14] DPO structure was formed in tandem reaction. Different DPO derivatives were synthesized by using different amines. 19 kinds of DPO derivatives were obtained when 20 amines reacted with ethyl glyoxalate. The structures and yields of DPO derivatives are listed in Table 1.

The synthesis of DPO was previously achieved by the multi-step reaction like other five member nitrogen heterocyclic rings.[10,11] but the entire synthesis process is extremely cumbersome and complicated because the by-product must be purified in each step.

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http://dx.doi.org/10.1016/j.bmcl.2014.12.010
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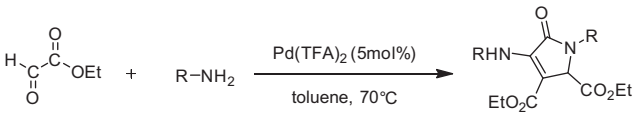


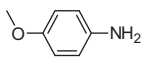
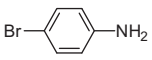
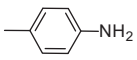
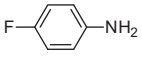
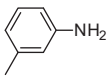
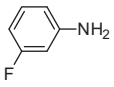
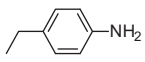
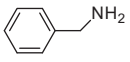
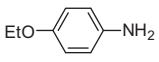
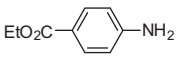
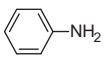
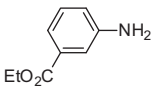
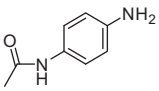
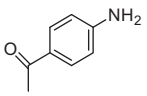
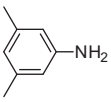
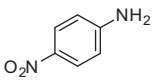
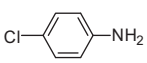
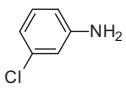
Figure 1. The synthetic route of dihydro-pyrrol-2-one.

We designed a new synthetic strategy in tandem catalytic system to achieve the diversity and complexity of products by adjusting the variety and portion of the substrates. Tandem reaction makes raw materials to form complex structure without isolation of the intermediates. It greatly reduces expenditure of solvent, energy and time, and is a promising method in the field of organic synthesis.

Based on low-cost raw materials, ethyl glyoxalate and amines are chosen to build DPO structure. The mechanism is that ethyl glyoxalate interacts with equivalent amine to form acyl imines by dehydration. At the same time, palladium combines with ethyl glyoxalate to be a chelate by oxidative addition. Finally, acyl imines and the chelate continuously react to get DPO through cross coupling or cyclization.[15,16] The reaction mechanism is illustrated in Figure 2. Arylamines have good adaptability with either electron-withdrawing groups or electron-donating groups such as methyl, alkyl, alkoxy, halogen, ester, acetyl aniline and ketone, and can react with ethyl glyoxalate to reach high yield. However, arylamines with *meta*-substituted group have slight decrease in yield than with *para*-substituted group, it may be attributed to steric hindrance on amino nitrogen atom. *p*-Nitro aniline cannot generate the desired product, it may be due to the strong electron withdrawing effect of *p*-nitro inhibiting the formation of imine.[17]

DPO derivatives exhibit different biological activities if there is change of substituent in γ-lactam ring, especially the 3-amino substituted compounds because its enamine structure can be further functionalized.[18] For example, α,β-unsaturated lactam structure of DPO has Michael addition reaction with stable carbon anions, nitrogen nucleophiles and Gilman reagents, which are owing to the presence of a double bond in the ring prone to epoxidation and hydroxylation.[19] In addition, DPO dihydropyrrolo ring can be oxidized to be pyrrole, and reduced to be pyrrolidone, which

Table 1
Yields of DPO derivatives in ethyl glyoxalate reaction with amines

Sample no.	Amine	Yield (%)	Sample no.	Amine	Yield (%)
1		67.5 ± 3.4	11		73.7 ± 2.6
2		61.0 ± 2.4	12		71.5 ± 3.1
3		36.3 ± 2.0	13		36.3 ± 3.6
4		70.5 ± 3.6	14		40.8 ± 2.5
5		63.2 ± 3.3	15		57.7 ± 4.5
6		57.5 ± 4.6	16		69.7 ± 3.7
7		38.2 ± 2.9	17		26.0 ± 3.8
8		34.8 ± 2.3	18		0.0 ± 0.0
9		78.2 ± 2.8	19		55.3 ± 4.9
10		64.2 ± 3.5	20		34.8 ± 3.9

Yield was expressed as mean ± standard deviation ($n = 5$).